SUMMARY STATEMENT

(Privileged Communication)

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Application Number: **1 R01 AI069146-01A2**

Principal Investigator

ALFANO, JAMES R PHD

Applicant Organization: UNIVERSITY OF NEBRASKA LINCOLN

Review Group: **HIBP**
Host Interactions with Bacterial Pathogens Study Section

Meeting Date: **06/13/2007**
Council: **OCT 2007**
Requested Start: **07/01/2007**

RFA/PA: **PA07-070**
PCC: **I2B**

Project Title: **Suppression of innate immunity by an ADP-ribosyltransferase type III effector**

SRG Action: **Priority Score: 115 Percentile: .9**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **10-No live vertebrate animals involved for competing appl.**

Project Year	Direct Costs Requested	Estimated Total Cost
1	250,000	363,101
2	250,000	363,101
3	250,000	363,101
4	250,000	363,101
5	250,000	363,101
TOTAL	1,250,000	1,815,506

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

1R01AI069146-01A2 ALFANO, JAMES

RESUME AND SUMMARY OF DISCUSSION: This application seeks to test the hypothesis that targets of the *Pseudomonas syringae* type III effector HopU1, a mono-ADP-ribosyltransferase (ADP-RT), will be components of innate immunity. These studies should bring new insight into suppression of plant innate immune responses and RNA regulation in innate immunity and is highly significant work. This revised application has been improved over the previous submission. The impact of this project and experimental progress of this talented and productive investigator are documented by the recent publication of his preliminary data in Nature. The aims of this project are exciting, focused and well thought out. There is particular enthusiasm for Aim 1, to determine the consequence of ADP-ribosylation on the function of the RNA-binding protein *At*GRP7, which was described as “the most interesting aim and should remain a top priority”. Overall, this application generates the highest levels of enthusiasm.

DESCRIPTION (provided by applicant): The eukaryotic innate immune system represents an important barrier that pathogens need to circumvent in order to cause disease. Several components of this system are conserved in eukaryotes. Recently, bacterial pathogen effectors that are injected into host cells by type III protein secretion systems (TTSSs) have been shown to be capable of suppressing innate immunity in eukaryotes. The bacterial plant pathogen Pseudomonas syringae is dependent on a TTSS to cause disease on plants. The P. s. pv. tomato DC3000 effector gene hopU1 resembles ADP ribosyltransferases (ADP-RTs) genes. These genes encode some of the best understood toxins in bacterial pathogens of animals (e. g., cholera toxin). Preliminary data within this proposal show that HopU1 is an active ADP-RT and that it ADP-riboslylates several plant proteins. Mass spectrometry determined that chloroplast and glycine-rich RNA-binding proteins acted as in vitro substrates for HopU1. These are novel substrates for ADP-RTs. Moreover, HopU1 has the ability to suppress several responses of the plant innate immune system in a manner that is dependent on its ADP-RT active site. An Arabidopsis mutant lacking one HopU1 substrate, AtGRP7, displayed enhanced susceptibility to P. syringae suggesting that it is a component of innate immunity. AtGRP7 is a glycine-rich RNA-binding protein, which suggests this pathogen targets proteins involved in RNA metabolism to suppress innate immunity. The central hypothesis of the proposed experiments is that AtGRP7 and perhaps other targets of the HopU1 ADP-RT type III effector are components of innate immunity. Several of the experiments seek to elucidate the role AtGRP7 plays in innate immunity using biochemical and molecular biological approaches. The P. syringae-Arabidopsis pathosystem is an excellent model to study the innate immune system because of the resources available, the similarities between innate immune systems between eukaryotes, and the cost efficient research. These experiments will contribute to a fundamental understanding of the molecular mechanism of bacterial pathogenesis and innate immunity.

The Specific Aims are the following: (1) Determine the molecular consequence of ADP- ribosylation on the function of AtGRP7 and elucidate the role this protein plays in innate immunity; (2) Identify additional substrates of HopU1 and verify their involvement in innate immunity; (3) Analyze the affect that HopU1 has on host-microbe interactions.

Project Narrative: Identifying the eukaryotic targets for the P. syringae HopU1 ADP-ribosyltransferase will contribute to our understanding of bacterial pathogenesis and will likely reveal important components of the innate immune system. One HopU1 target belongs to a large group of proteins called glycine-rich RNA binding proteins, which are not well understood, and this research will likely increase our understanding of these proteins. Because there are considerable similarities between the innate immune systems in plants and mammals we expect that our findings will be relevant to the mission of the NIH and be broadly interesting to researchers studying molecular mechanisms of bacterial pathogenesis and innate immunity.

CRITIQUE 1:

Significance: This is highly significant research applying the Pseudomonas syringae-Arabidopsis model system to genetically and biochemically dissect the function of HopU1 in the suppression of plant immune responses. The investigator has discovered that HopU1 is a bacterial toxin with ADP ribosyltransferase (ART) activity that inhibits programmed cell death (PCD) as well as innate immune responses triggered in infected plants. HopU1 ADP-ribosylates several plant proteins but RNA binding proteins (RBPs) appear to be the high affinity substrates. HopU1 also modifies proteins in animal cells suggesting that similar eukaryotic targets or pathways may be affected. The central hypothesis is that HopU1 is suppressing host defense responses by directly targeting and potentially inactivating host components involved in innate immunity signal transduction.

This work is novel. The impact and interest of this work is supported by the recent publication of the preliminary data in Nature. Importantly, we know very little about the cellular mechanisms and modes of regulation that control the innate immune system. The identification of a host RNA binding protein that is involved in pathogen defense, and is a target of a bacterial virulence factor is very exciting. Deeper insight into the area of RNA regulation in innate immunity is essential. This application provides a starting point for such knowledge.

Approach: The revised application has 3 aims and is well focused. The experiments are well designed and are expected to be successful. Again, the investigator paid close attention to the reviewer's comments and improved the application where needed.

Aim 1 is new and is focused to determine the consequence of ADP-ribosylation on the RNA-binding protein identified, AtGRP7. This is the most interesting aim and should remain the priority. AtGRP7 mutants will be studied to study the impact of ribosylation on RNA binding. Microarrays and RIP-chip studies will be performed to identify RNA targets. Protein-protein interactions will be done to identify players involved in AtGRP7 function. The investigator mentioned that AtGRP7 functions in circadian clocks. I find this intriguing and perhaps the investigator will discover new information relating eukaryotic clocks and innate immunity.

Aim 2 is to identify HopU1 substrates in vivo, and determine their phenotypes (knock-outs or downs). A sub-aim is to identify mammalian targets of HopU1. The investigator is still very interested in this and strongly believes that HopU1 may identify novel target in mammals. I am supportive of this. The sub-aim priority seems appropriate at this stage.

Aim 3 is now the previous Aim 1, focused to identify addition innate immune responses altered by HopU1. This is an important physiological study to phenotype the interaction in more detail. This information will be used to monitor innate responses studied in Aims 1 and 2.

Innovation: Most of approach and methodologies are standard, but are solid and well thought out. The novelty is that the investigator has discovered novel host targets (RBPs), optimized assays and tools to study ART activity in plants, and is focused on characterizing the role of RBPs in host-microbe interactions.

Investigators: The principal investigator is an excellent, mid-career investigator. He has never had an R01, but has none-the-less been able to produce a steady stream of important papers. Most notably, this investigator was one of the first in the field to show that TTSS effectors suppress host innate immunity. This was first taken with skepticism… but now the field accepts the insight and is scurrying to determine mechanism. The investigator has already done that with this preliminary data… published in Nature.

Environment: Excellent.

Overall Evaluation: Outstanding.

Biohazards: Appropriate containment and disposal of pathogenic bacterial and fungal strains and transgenic plans is necessary. The investigator is well aware of this.

CRITIQUE 2:

Significance: The investigator has uncovered an entirely novel set of host targets (RNA binding proteins) that are impacted by type III-secreted effector, and has shown that they are relevant to the host innate immune response. His group's article in Nature about this work, published a few weeks ago, demonstrates the high regard in which this work is held, and importantly, that paper is significant not only for what has been learned but also for what it opens up for further study. Dr. Alfano now proposes to understand how the effector is acting on its substrates (preliminary work has already advanced this effort significantly) and (perhaps more excitingly) to understand how the effector substrates impact host immune systems. Given the exciting new roles that are being discovered for RNA modulation (e.g. miRNAs), investigation into what makes RNA binding proteins a preferred target for pathogen TTSS effectors is highly significant work.

Approach: The approach details are appropriate to the task at hand. For example, modulation of specific host RNAs during pathogenesis is hypothesized, making the proposed microarray expression profiling and RIP-chip experiments especially appropriate. It would be inappropriate to worry that the investigator is not an old hand at binding constant determinations, microarrays, RIP-chip or other proposed methods because the investigator has proven his ability to bring methods into his lab and/or to engage appropriate collaborators, to carry out experiments in a critical fashion using proven methodologies that are new to his lab.

The fact that they are already well along in finding out (since application submission) how HopU1 disables AtGRP7 is to their credit, not detriment, and much remains to be done with Aim 1. The "discovery" flavor of the microarray, RIP-chip and interaction cloning are excellent for this project (precisely appropriate at this juncture in our understanding).

The first part of Aim 2 is very solid – the investigator has already shown competence and success in the area of identification of in vitro substrates of HopU1. The "low-hanging fruit" may have already been obtained, but solid approaches are outlined to identify the less prominent substrates. The second part of Aim 2.d. (identification of novel plant substrates in vivo) may or may not work and in my opinion is the one significant weakness of the application. However, the success of the application does not hinge on this experiment because the investigator already has discovered a very interesting and relevant set of players with the RNA binding proteins that fully justify the project and will keep them busy. Aim 2e (animal substrates) is now presented as a less central aim in this revised application, and it is excellent that it will be pursued at this level. It could yield interesting results that feed back into biologically relevant Arabidopsis and mammalian experiments.

Aim 3 – This is a very standard yet appropriate set of experiments, to identify the aspects of plant immune responses that are impacted by the RNA binding protein (and by effector-mediated inhibition of that protein). This section could have been written more deeply (proposing more different threads of analysis), but it is clear that the investigator plans simply to do the appropriate experiments that are well-established in the plant immunity discipline, so this deficiency is a matter of grantsmanship and not of scientific merit.

Innovation: The innovation is not in the methodologies – the vast majority of those are time-tested. The innovation is in the novelty of the effector and its targets (see significance, above) – because no one else in the plant world seems to be doing work with this type of immune system component, and only perhaps one or two other groups are starting to work on this topic in the animal pathogenesis field.

Investigators: Dr. Alfano for the last decade has consistently been at the forefront of his discipline (effector biology and virulence of plant-pathogenic bacteria). He is an acknowledged expert in the

broader fields of bacterial pathogen effector biology (all hosts) and in plant-microbe interactions. He understands how to do quality work, and how to work on areas that are path-breaking rather than confirmatory. He has shown himself to be in the top echelon of an elite group of “plant researchers” with his recent Nature paper.

Environment: The University of Nebraska Lincoln has the resources and technical expertise necessary to complete most of the proposed work, and excellent off-campus collaborators have been engaged where needed. Dr. Alfano seems quite able to attract and develop talented lab members. He and other peers at Nebraska produce a continuous stream of top-shelf findings.

Overall Evaluation: This is exciting work in that straightforward, high-probability experiments are proposed on an exciting new aspect of host-pathogen interactions (effector targeting of host RNA binding proteins) that is now of proven relevance. Given the investigator’s track record of leadership and impact and the fact that this is an improvement on two previous versions of this application, this application rates as extremely strong.

Budget: Appropriate.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW ADMINISTRATOR TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

MEETING ROSTER

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June 13, 2007

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* Temporary Member. For grant applications, temporary members may participate in the entire meeting or may review only selected applications as needed.

Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.